Application No. 09/694,088
Title: GLYCEROL AS A PREDICTOR...

Inventors: Daniel Gaudet, et al.



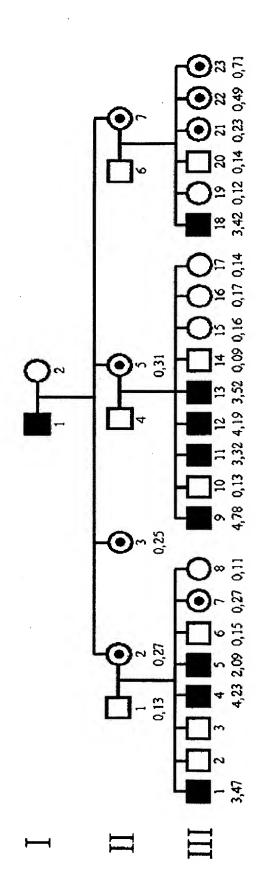
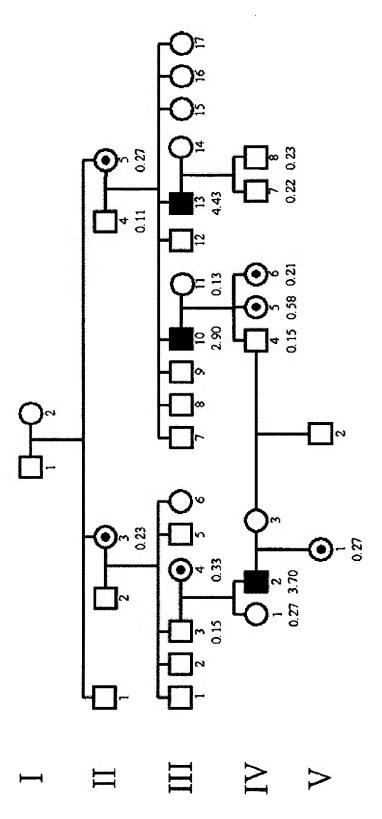
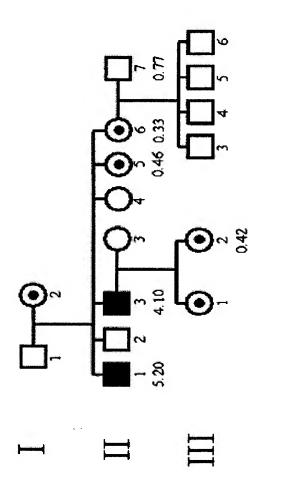


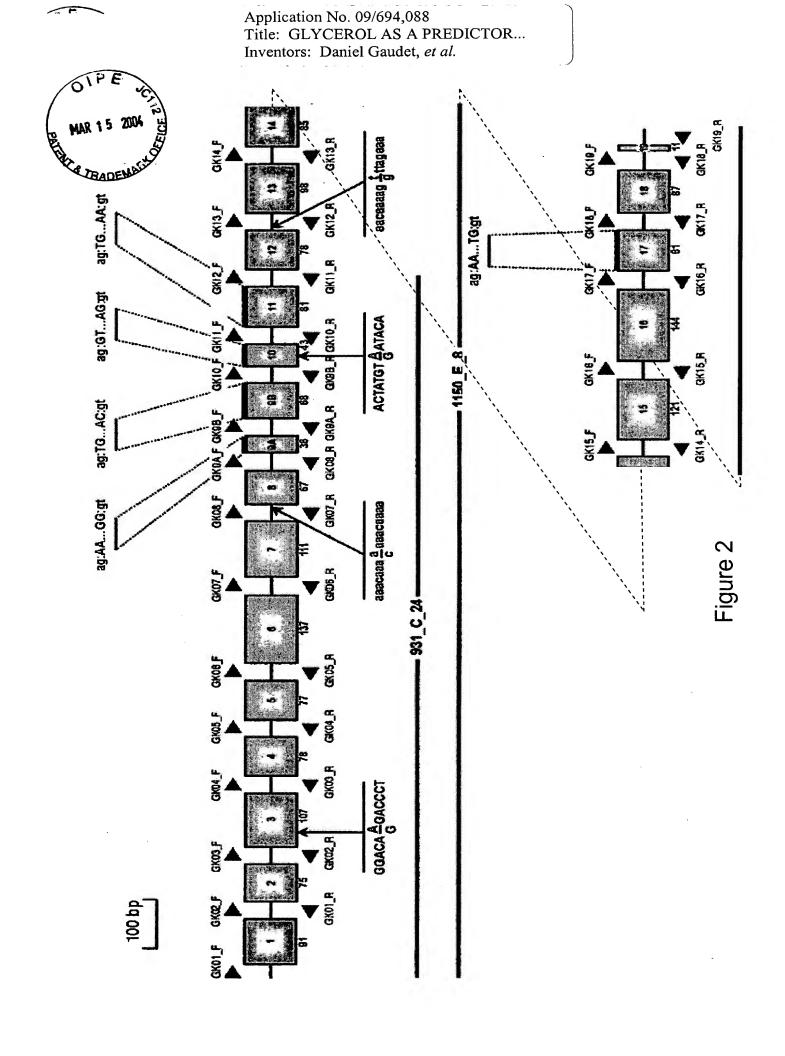
Figure 1A











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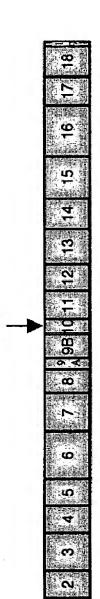


Figure 3A

TAT GGA ACA GGA TGT TTC CTA TGT  $\frac{A}{G}$ AT ACA GGC CAT AAG O

SEQ ID NO: 11 SEQ ID NO: 12 SEQ ID NO: 8 SEQ ID NO: 9 SEQ ID NO: 10 **SEQ ID NO: 6** SEQ ID NO: 7 FOIGQAKNTYGTGCFLLCTGTGHKCVFSDHGLLTTVAYKLGR **FQIGQAKNTYGTGCFLLOTGHKCVFSDHGLLTTVAYKLGR** FODGQAKNTYGTGCFLLCATGHKCVFSEHGLLTTVAYKLGR FODGOAKNTYGTGCFLLONTGHKCVFSEHGLLTTVAYKLGR VKEGMAKNTYGTGCFMLMMTGEKAVKSENGLLTTIAC--GP VEPGOAKNTYGTGCFLLM#TGDKAVKSTHGLLTTIAC--GP FERGMIKNTYGTGAFIVM TGEEPOLSDNDLLTTIGY--GI **3K N288D Mutant** glpk\_human glpk\_mouse glpk\_pseae glpk\_entca glpk\_ecoli glpk\_rat

SEQ ID NO: 13

VHAGQAKNTYGTGCFMLIHTGNKAITSKNGLLTTIACNAKG

YKPGAAKCTYGTGCFLLYMTGTKKLISQHGALTTLAFWFPH

FEEGMGKNTYGTGCFMLMNTGEKAI KSEHGLLTTIAW--GI

glpk\_bacsu

lpk\_yeast

lpk haein

**SEQ ID NO: 14** 

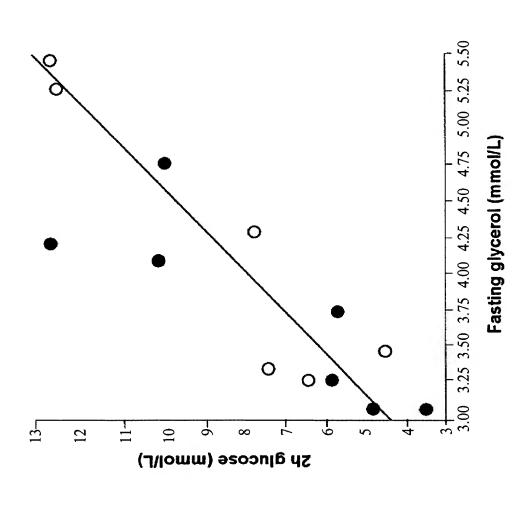
270

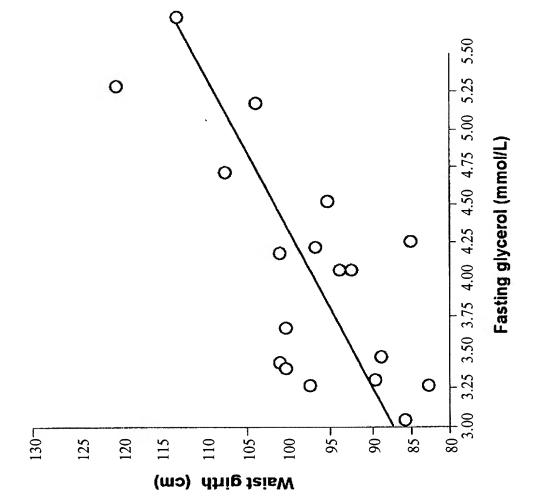
SEQ ID NO: 15 SEQ ID NO: 16 **SEQ ID NO: 17 SEQ ID NO: 18** TEPGMVKNTYGTGCFVTMTGDKPTLSKHNLLTTVAWOLEN vepamykntygtgcfmlmignelkysohnllitvamolen **DRPGLLKCTYGTGAFLVANTGQTVTRSQHRLLSTVAMTQTN** FEPGMVKNTYGTGSFIVM/TGEEPQLSKNNLLTTIGY--GI glpk\_mycge lpk\_entfa

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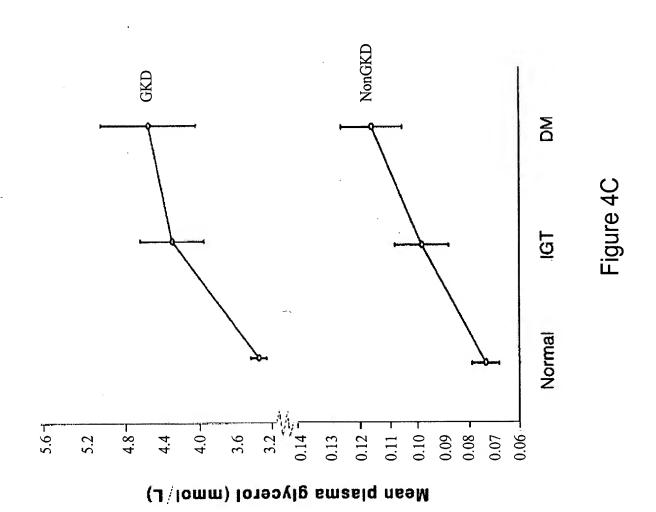
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0.35

Fasting glycerol concentration (mmoVL)

0.25

0.7

0.6

0.55

Figure 5

Title: GLYCEROL AS A PREDICTOR...

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poly: A/G

location:13th base of exon 3

ATGCCTTCTTTTGTCAAAGATGGGTGGAACA[A/G]GACCCTAAGGAAATTCTACAT
TCTGTCT SEQ ID NO: 1

CAA vs CAG ==> silent

poly: A/C

location:17th base of intron 8

TAATGGTAAAAAACAAACAAA [A/C] AAACAAAAAACACACCAAAAAAACCAA

SEQ ID NO: 2

poly: A/G

location: 29th base of exon 10

TTCATTCTCCCTTCAACCATAGGTATGGAACAGGATGTTTCTTACTATGT[A/G]AT ACAGGCCATAAGGTtGGTTTTTAATAAAAATGATTAAGTCA SEQ ID NO: 3

AAT vs GAT ==> N to D

poly: G/T

location: 22nd base of intron 12

GAAATTGGTGAGTGTTCTAACAAAAG [G/T] TTAGAAAATCTGAAAAATGACACA

TTTCA SEQ ID NO: 4



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SEQ ID NO: 5

# Exon 1:

## Exon 2:

# Exon 3:

CAATGCCTTCTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACATTCT GTCTATGAGTGTATAGAGAAAACATGTGAGAAACTTGGACAGCTCAATATTGATATTTCCAA CATAAAAGGTATTTTAGTAGAATATTTTACCCACA

## Exon 4:

TGTAAAACGACGGCCAGTTGAGAGCTGTTTTCCTGAAGTAGTTCCTACTTGTTAAATTTTTG ACTTCCTTCTGTTTAACTTTCTCTTTAAAGCTATTGGTGTCAGCAACCAGAGGGAAACCACT GTAGTCTGGGACAAGATAACTGGAGAGCCTCTCTACAATGCTGTGGGTAAGCTGTCATGCAT GGATGTCAAATGTAGGGCCTTTCTTCACATTGCAA

#### Exon 5:

# Exon 6:

Title: GLYCEROL AS A PREDICTOR...

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xon 7:

TGTAAAACGACGCCAGTTGTGCTCTGCTGATTATGACCCTTAACAATATGTAAATTAAATT GCCAATAAGTACAAATTTAACCTGATTTTTTTTACTCTGCCTAGAGTTTGACAGGAGGAGTCA ATGGAGGTGTCCACTGTACAGATGTAACAAATGCAAGTAGGACTATGCTTTTCAACATTCAT TCTTTGGAATGGGATAAACAACTCTGCGAGTAAGTTCTGTTTTGCTCTAAATATAGTTTTCC CAATACACTACCTATTTAAACCGAAATCTTAATATTTCAGATGTCAGTGGAGCA

## Exon 8:

# Exons 9A and 9B

## Exons 10 and 11:

TTATTTGCTTTCAATAAAATTGTCTTCTATTCATTCTCCCTTCAACCATAGGTATGGAACAG GATGTTTCTTACTATGT [A/G] ATACAGGCCATAAGGTTGGTTTTTTAAATTAAAAAATTGA TTTAAAAGTCTAAGTTCATCTAAATAATGCTTGAACATAATTTACTATTAAACAACTTTTAG TCTTTAGCTTTACTTAATCTTTATCAGGGTTTAATTTAGAGCTCAATACAAAATTTGAATC GTTCTAATAAGAACCATTTTAGACTCTTTGAATTTTATATGTGTGTTTTTAATTGTGCTGGG GGGAAATCTAGACTGAGACCTCATCAAATTCTTAATGCAAATCTAATTTGAAACAAGGAATA ATTTTCTGATCATGGCCTTCTCACCACAGTGGCTTACAAACTTGGCAGAGACAAACCAGTAT ATTATGCTTTGGAAGTAAGTTCTTTTTAATCAATATGGATAATATGACAAACATTCAAAGCT AATAAAAATCACAGAGTTTTCTAACACTTTTCTGGTAAATCTTAATACAGAGGACTCAAAAA GTTCTGCTTTCTTGGCATTTGATTGAGTTGAAGGAACCTGAAACTGATCTGGGTGTCAGGAC TCACAGGAGACCTTGATTAGATTGGTTCCTCAGTTCTTATGCCAATTAATCATGTCACCTTA TGCTCCAGTGTTCCAAAGAGAACCCTGGGCACAAATAGGCAGAACAACTCTCTTCACTTGTC CCACTTATCACTGGAAACATTTGTTTCAAACATTTTTGTATGTTATAGTAGGAATATGCCAG CCTAAGCCTATA

Figure 7B

Title: GLYCEROL AS A PREDICTOR...

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Exon 12:

#### Exon 13:

 ${\tt CCAGTTGTGTTTTTTTTTTTTTTTTTATGTTAAAAAACTTGCTAAAGAAGTAGG}\\ {\tt TACTTCTTATGGCTGCTACTTCGTCCCAGCATTTTCGGGGGTAATATGCACCTTATTGGGAGC}\\ {\tt CCAGCGCAAGAGGGTAAGTATTGAAAATATGGAGTGCTTTTTGGGGATCTTGATTTAT}\\$ 

# Exons 14 and 15:

TGTAAAACGACGGCCAGTTGATTATGTCCAATTTTCTCTTCCTGGACATTTCTGTCTACCAA ATTTGACCTTTTCATATTTGAGATATTTCAAATTGATTGGTTTATATCATTCTAATCTGAAA TGCTTTTGCTGCATTAGAAGCTGTTTGTTTCCAAACTCGAGAGGTAACAAATATGGGCCTGT TTTCTTGTACTTAGTTCACTTTTATCACTCTTAAGTTATATGTTAACACCCCGAGATTTATTC AGTACTGAAAATGTAGTTAATCAAATATTAAGGCTGCCTAAATACTAATCTAAATATAAGCA GGGTTTTCCCCCTTTTTCCAGCTGTCATTACCTTCTAAGTTCCTGTTCCCTGTCAGGCACTG GGAAATTTATGGTTGTGGGGAGGCTGAGTGGCACACATTAGGCAAAGGAAACAGCACAAACA TAGGCATCaAGGCAGAAAAACAGGGTGCAAAATAGAGTTGTATAGCTTAGCTGAATATCAAG GTGAATGCAGAGGTGTAGTGAGAGAAAAGGTTGGCTGTGACCAGATCAAAGAGGGCTTAGAA GACCAGAATAAGAAGTCTCAATTTATTCCATAGGCTCTTGGAAGCTCTTGAGAGTTTCTGAG TGGAGGATTGCCATTTTCAGAGATGTTACTATGAAATAGATTTATAACATTAATTGCACTGG TTTATTTAAGATTTTGGATGCCATGAATCGAGACTGTGGAATTCCACTCAGTCATTTGCAGG TAGATGGAGGAATGACCAGCAACAAAATTCTTATGCAGCTACAAGCAGACATTCTGTATATA CCAGTAGGTTAGTAAGTCTTCATTCCTTTAAACTCCCAGAGTAATGTTTCTTGTGGAATAAC TAGTTCTTTGGG

#### Exon 16:

#### Exon 17:

Figure 7C

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Exon 18:

#### Exon 19:

AAAATTACTGGCTTAAATGGAAATGATGCTTCTTATTCTGTATGTTCCCATGAAAGTGAAAC TTAAAAAAAAATTCATGATTAGGGTTTCATGAAAAGGCCTTGTTTCTATGAAAATTGAGAC AGGTTGCATCTCTAAGCTAAAAGATGGGCTATGTGTCTAGAGTCTTAGACTTCTAAAATG CATGTGGTCACTATATGTAGGTTATCTCTTCGGTGACATACACTGCAATTTGAGAGGGCTGG AAATTGTTTGCCTTGGTAAACGATTAGCAACAGTGGCAATATTTTGTTAATTTTGGAATTGGC CCTGTTTGTTGCATTTTAATTGTGAGGCATGATTTAGAAATCATATGGACTTTCTAGCTTAA TAAATGATTGAATCATCTGCATTGCTTTAACTCCTGAATTGTATGCATGTATTATTGACATA TATGGTTTTTGTTCCCCATTTCAGGTATTCCATAAAACCTACCAACTCATGGATTCCCAAGA TGTGAGCTTTTTACATAATGAAAGAACCCAGCAATTCTGTCTCTTAATGCAATGACACTATT CATAGACTTTGATTTTATTATAAGCCACTTGCTGCATGACCCTCCAAGTAGACCTGTGGCT TAAACATCCACAGTTAAGGTTGGGCCAGCTACCTTTGGGGCTGACCCCCTCCATTGCCATAA CATCCTGCTCCATTCCCTCTAAGATGTAGGAAGAATTCGGATCCTTACCATTGGAATCTTCC ATCGAACATACTCAAACACTTTTGGACCAGGATTTGAGTCTCTGCATGACATATACTTGATT AAAAGGTTATTACTAACCTGTTAAAAATCAGCAGCTCTTTGCTTTTAAGAGACACCCTAAAA GTCTTCTTTCTACATAGTTGAAGACAGCAACATCTTCACTGAATGTTTGAATAGAAACCTC TACTAAATTATTAAAATAGACATTTAGTGTTCTCACAGCTTGGATATTTTTCTGAAAAGTTA TTTGCCAAAACTGAAATCCTTCAGATGTTTTCCATGGTCCCACTAATTATAATGACTTTCTG CTTTGTATGTATAACATACATGCCTATATATTTTATACACTGAGGGAGCCCATTTATAAATA AAGAGCACATTATATTCAGAAGGTTCTAACAGGG



Table 1. Characteristics of Carriers of the N288D GK Gene Mutation and of Their Unaffected Relatives

		Men			Women	
	N288D carriers	Unaffected relatives	d	N288D carriers	Unaffected relatives	d
Z	18	. 18		14	14	
Age (years)	46.4±14.2	42.0±18.8	0.32	44.9±13.5	43.7±17.8	0.87
Uncorrected triglyceride (mmol/L) <sup>(1)</sup>	6.26±1.13	2.05±0.54	<0.0001	2.84±1.20	1.30±0.65	0.0002
Glycerol (mmol/L)	3.99±0.71	$0.10\pm0.04$	<0.0001	$0.54\pm0.14$	$0.10\pm0.02$	<0.0001
Corrected triglyceride (mmol/L) <sup>(1)</sup>	2.27±0.75	1.95±0.53	<0.0001	2.31±1.22	$1.19\pm0.67$	0.03
Free fatty acid (mmol/L)	0.77±0.22	0.57±0.25	0.01	$1.29\pm0.35$	0.76±0.17	0.0004
Fasting glucose (mmol/L)	5.2±0.74	4.8±0.31	0.13	5.0±0.7	4.6±0.3	0.10
2h glucose following OGTT (mmol/L)	7.9±3.1	5.8±1.6	0.02	7.0±6.1	5.0±2.1	0.04
Fasting insulin (mU/L) <sup>(1)</sup>	13.3±14.0	15.1±14.8	0.62	12.2±13.1	9.0±3.4	09.0
Waist girth (cm)	97.7±9.3	88.1±12.3	0.01	88.5±3.8	79.8±5.8	0.03
Body mass index (kg/m²)	27.9±4.1	24.9±3.9	0.03	28.1±5.5	23.1±2.3	0.001
%Total body fat	27.1±7.2	22.9±7.6	0.01	46.8±8.1	33.9±11.3	0.001
(1) Geometric mean, p after log transformati	ion.					

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Table 2. Fasting plasma glycerol concentration (mmol/L) in the initial cohort of 1056 individuals, by risk factor of glucose intolerance and diabetes mellitus

	Glycerol		
	No.	geometric mean $\pm$ SD	p
Gender			
men	717	$0.065 \pm 0.081$	
women - premenopaused	137	$0.071 \pm 0.093$	< 0.0001
- menopaused	202	$0.099 \pm 0.085$	
Age (Y)			
<50	486	$0.071 \pm 0.082$	
50 - 60	408	$0.076 \pm 0.106$	0.0015
>60	165	$0.083 \pm 0.053$	
Fasting glucose (mmol/L)			
< 5.2	449	$0.068 \pm 0.080$	
5.2 - 5.9	336	$0.070 \pm 0.090$	< 0.0001
6.0 - 6.9	271	$0.090 \pm 0.100$	
Fasting insulin (UI)			
<15	637	$0.067 \pm 0.082$	0.02
≥15	419	$0.086 \pm 0.101$	
2 hours glucose (mmol/L)			
<7.8	572	$0.062 \pm 0.071$	
7.8 - 11.0	283	$0.081 \pm 0.101$	< 0.0001
ε11.1	201	$0.102 \pm 0.110$	
Triglyceride (mmol/L)			
≤ 2.2	389	$0.057 \pm 0.062$	< 0.0001
>2.2	667	$0.082 \pm 0.103$	
Free fatty acid (mmol/L)		-	
< 0.6	589	$0.066 \pm 0.054$	< 0.0001
ε0.6	467	$0.111 \pm 0.112$	
Body mass index (kg/m2)		<u>-</u>	
≤ 27	428	$0.060 \pm 0.087$	< 0.0001
>27	628	$0.079 \pm 0.097$	0.0001

p value from a one-way ANOVA

Figure 9



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Table 3. Multivariate analysis of the relationships of fasting plasma glycerol concentration with impaired glucose tolerance (2h glucose 7,8-11,0 mmol/L following a 75 g oral load) before and after adjustment for covariates identified in

-	Model 1	Model 2	Model 3	Model 4
	17104011	1/104012		1,10401 1
Glycerol (log)				
ß	1.75	1.62	1.46	0.77
Odds ratio	5.76	5.42	4.33	2.46
p	< 0.0001	< 0.0001	< 0.0001	0.01
Triglyceride (log)				
ß		0.54	0.35	0.12
Odds ratio		1.75	1.42	1.12
p		0.02	0.11	0.63
Body mass index (	kg/m²)			
ß	<i>5</i> /		0.10	0.05
Odds ratio			1.10	1.05
p			<0.0001	0.01
Fasting insulin (lo	g)			
В				0.57
Odds ratio				1.31
p				0.39
Fasting glucose (m	mol/L)			
ß				1.13
Odds ratio				2.65
p				< 0.0001
Free fatty acid (lo	g)			
ß	•			1.62
Odds ratio	,			4.33
p				0.007

Odds ratios are expressed as the increase in the risk of 2h glucose 7.8-11.0 mmol/L following a 75 g oral load, associated with a 1-SD increase in the variables studied. B denotes the standardized estimate which is the parameter estimate of each variable in the multivariate logistic model. All models included age and gender as covariates. Otherwise, only the variables included in each model are shown. Subjects with severe hyperglycerolemia due to the N288D mutation in the